Chapter 1
Classic Circadian Characteristics: Historical Perspective and Properties Relative to the *Synechococcus elongatus* PCC 7942 Model

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Abstract The purpose of this chapter is to introduce the basics of circadian biology relative to the cyanobacterial model system. It is meant to define the terms, characteristics, and rules that pertain to the study of circadian biology in the context of the cyanobacterial systems used to elucidate the mechanisms by which the prokaryotic circadian clock functions. In addition, its purpose is to serve as a conduit to the chapters in this book, which comprehensively review our most recent understanding about each of these canonical characteristics in the *Synechococcus elongatus* PCC 7942 model system as well as other cyanobacterial and prokaryotic systems.

1.1 Introduction

1.1.1 Overview

Our planet rotates about its axis every 24 h, which exposes the majority of plants and animals that inhabit the earth to sidereal fluctuations of light and temperature. This daily change in light and dark was a strong selective force (for those organisms that are subject to it) to devise physiological mechanisms with which to respond to, or better yet predict, when these daily changes were going to occur. As a result of this pressure, organisms have evolved internal timing mechanisms to anticipate the daily variations in light and temperature; this anticipatory behavior provides a selective advantage to the organism (DeCoursey 1961; Ouyang et al. 1998; Michael et al. 2003; Woelfle 2004; Johnson 2005).

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This daily clock phenomenon was termed "circadian" in 1959 by Franz Halberg using the Latin terms *circa* for "about" and *dies* "day". Therefore circadian phenomenon pertain to biological activities with a frequency of one activity cycle every 24 h (Halberg et al. 1977). The purpose of this chapter is to introduce the basics of "circadiana": to define the numerous terms, characteristics, and rules that pertain to the study of circadian biology in the context of the cyanobacterial systems that have been used to elucidate the mechanism by which the prokaryotic circadian clock functions. In addition, its purpose is to serve as a conduit to the chapters in this book, which comprehensively review the most recent understanding about each of these canonical characteristics in the *Synechococcus elongatus* PCC 7942 model system as well as other cyanobacterial and prokaryotic systems.

1.1.2 Historical Perspectives

Investigations into the mechanism that organisms use to relate and respond to diurnal fluctuations in light and temperature have been undertaken at least as early as the 1700s. One of the earliest reports that correlates behavior with specific times of day came from the French astronomer Jean-Jacques d'Ortous deMairan, who made the observation that the leaves of heliotrope plants move in response to changes in light. Even more importantly, he recognized that these leaves would continue to move in the same pattern when kept in constant darkness (DD), generating the first evidence that a behavioral activity could be regulated by an internal mechanism of the plant, and not a result of environmental light and dark cues (deMairan 1729). During the same period, the Swedish botanist Carl Linneaus developed his *horologium florae* or "flower clock," which could be used to tell the time of day based upon when particular plant species would flower (Freer 2003).

The modern field of chronobiology, or the study of biological timing processes in living things, was initiated in the mid-1950s by Colin S. Pittendrigh and Jürgen Aschoff. They were instrumental in defining and organizing the principles of a circadian system that mapped the course for circadian research, and these rules still hold true to the present time (Aschoff 1960, 1981; Pittendrigh 1961, 1981). While the characteristics and principles of circadian biology were being brought to bear by early circadian biologists, a particular question of interest was whether circadian activity was a learned behavior in organisms or had a genetic basis. The work of Erwin Bünning in 1935 alluded to the answer by providing evidence that period length was heritable in bean plants (Bünning 1935); however, it was not until the early 1970s that the first evidence for a genetic basis to circadian activity was brought to light by two independent groups working in fruit flies and fungus. Ronald Konopka and Seymour Benzer isolated *Drosophila melanogaster* mutants that had altered eclosion and activity rhythms. Each of the mutations was complemented by one genetic locus, termed the *period* gene (Konopka and Benzer 1971). Soon after, Jerry Feldman and Marian Hoyle identified the *frequency* gene, which was shown to be essential for rhythms of asexual spore formation in *Neurospora crassa* (Feldman and Hoyle 1973).

The study of circadian clocks and rhythms was sequestered to eukaryotic models as historical circadian dogma dictated that nuclear structure, intercellular communication, and generation times longer than 24 h were required for rhythmic activity – characteristics that are lacking in prokaryotic cells and, at least in part, in unicellular eukaryotes (Edmunds 1983; Kippert 1987). However, in the 1980s, several lines of evidence were emerging to contradict the "eukaryocentric" circadian requirements. The cyanobacteria are a large and diverse group of micro-organisms that are typically photoautotrophic and diazotrophic, and are responsible for a vast majority of the carbon and nitrogen fixation in the environment (see Chap. 2; Garrity 2001). Within several different cyanobacterial species, circadian activity in nitrogen fixation, amino acid uptake, and cell division were identified (see Chap. 3; Grobbelaar et al. 1986; Mitsui et al. 1986; Sweeney and Borgese 1989; Huang et al. 1990; Chen et al. 1991; Grobbelaar and Huang 1992; Schneegurt et al. 1994). While the physiological evidence drastically changed the manner by which scientists thought about circadian biology, a good model system for prokaryotic circadian research was lacking. Ultimately *S. elongatus* PCC 7942 became the model of choice in part because of the vast amount of molecular tools available in this strain (see Chap. 4; Golden 1987; Golden 1988; Kondo et al. 1993, 1994; Ishuira et al. 1998; Andersson 2000).

1.2 Properties of a Clock-Controlled Rhythm

Regardless of the model system one is using to understand the circadian process, the underlying mechanisms achieve a similar goal: maintain an internal, 24-h time. A circadian clock system is defined as an endogenous mechanism that allows an organism to temporally regulate biological activity as a function of the 24-h day. Such biological activities that are regulated by the circadian clock are therefore coined circadian rhythms (Pittendrigh 1981; Edmunds 1983; Dunlap et al. 2004; Koukkari and Sothern 2006). The rhythmic nature of daily activity can be described by three terms that correspond to the characteristic descriptions of a waveform: period, phase, and amplitude.

1.2.1 Period

The period of a rhythm is defined as the duration of one complete activity cycle (Fig. 1.1). Therefore, a circadian period would be an activity that completed its cycle (with a frequency of approximately 1) over a 24-h period of time (Dunlap et al. 2004; Koukkari and Sothern 2006). When measured under constant conditions (see Sect. 1.3.1) the period is defined as the free-running period (FRP),

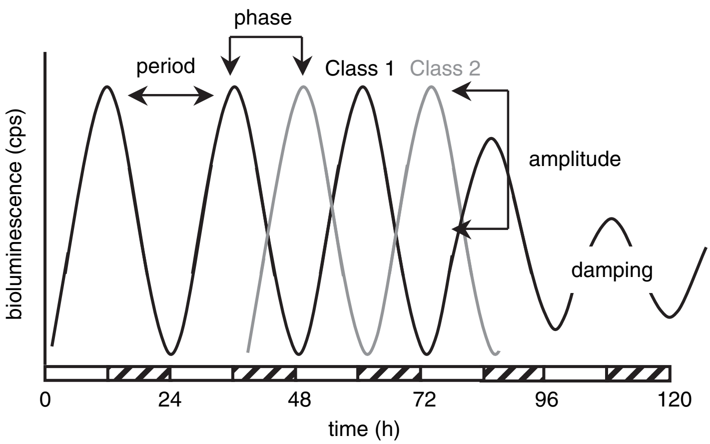

Fig. 1.1 Properties of a circadian activity rhythm as measured in *Synechococcus elongatus* PCC 7942. The traces depict levels of bioluminescence in counts per second (*cps*) over time (*h*) from two representative *S. elongatus* luciferase reporter strains maintained in constant conditions. Alternating *open* and *hatched* bars on the abscissa represent subjective day and subjective night, respectively. Period is defined here by peak-to-peak activity duration over approximately 24 h. Phase is defined here as the time when peak activity is reached. In the *S. elongatus* model, two phases are typically described: Class 1 (*black*) peaks at subjective dusk, while Class 2 (*gray*) peaks at subjective dawn. Amplitude is defined as the magnitude of the oscillation from the mean, where damping is a general trend whereby there is a decrease in rhythmic robustness over time under constant conditions

represented by τ (the Greek symbol *tau*). Observable, and therefore measurable, rhythms of a circadian timing system are not easily measured in bacteria due to their microscopic size and lack of obvious overt behaviors. Therefore, in *S. elongatus* PCC 7942, cyanobacterial promoters were engineered to produce the LuxAB luciferase proteins, as well as their necessary substrates (LuxCDE), from *Vibrio fischeri* and *V. harveyi* respectively, for bioluminescence as an easily measurable and quantifiable output (Kondo et al. 1993). While the average period for circadian rhythms in *S. elongatus* PCC 7942 is approximately 24–25 h (Kondo et al. 1993; Ishiura et al. 1998), the form or shape of the activity rhythm can vary considerably depending upon the promoter used to drive expression of *luxAB*. Waveform patterns of gene expression have been shown to be symmetrical sine curves, asymmetric, saw-tooth, or step-like in form (Liu et al. 1995).

1.2.2 Phase

The phase of an activity rhythm is defined as the instantaneous state of an oscillation within a period (Fig. 1.1; Dunlap et al. 2004; Koukkari and Sothern 2006). For example, the highest point of any rhythmic activity would be defined as the peak of activity (trough for the lowest). These peaks (or troughs) of activity can be used as reference points for determining at what point a particular activity occurs the most (or least) during a 24-h day. The majority of genes expressed in a circadian manner, as measured by random promoter:luciferase fusions in *S. elongatus* PCC 7942, were categorized into a number of different classes with the majority of the genes falling into either Class 1, where activity peaked at subjective dusk (the time in constant light, LL, that corresponds to dusk of the entraining light/dark, LD, cycle), or Class 2, where activity peaked at subjective dawn (the time in LL that corresponds to dawn of the entraining LD cycle; Liu et al. 1995).

1.2.3 Amplitude

The amplitude of a rhythm is defined as the magnitude from the mean activity level to either the peak or to the trough of activity (Fig. 1.1; Dunlap et al. 2004; Koukkari and Sothern 2006). Amplitude is an obvious requirement of a cyclic activity, but it is much more difficult to quantify and interpret than the period or phase of a rhythmic behavior, particularly in the cyanobacterial system. Typically, cultures of cyanobacterial cells (in lieu of individual cyanobacterial cells) are measured for circadian activity. Therefore, careful consideration of the number of cells being measured, the innate differences in the particular promoter driving expression of the reporter, and the level of substrate available for luciferase could each affect the measurement of the amplitude (Kondo et al. 1993; Andersson et al. 2000). Additionally, damping, or a decrease in rhythmic activity over time, can confound amplitude measurements; however, this has not been extremely problematic in the *S. elongatus* PCC 7942 model system, as robust rhythms have been measured for over two weeks in constant conditions (Golden and Canales 2003).

1.2.4 Time

The period, phase, and amplitude are all characteristics of activity patterns that are measured over time. When considering time in a circadian system, there are important distinctions that must be noted. Standard clock time is measured by mechanical or atomic clocks that are used to determine time of day with midnight placed in the middle of the dark and noon when the sun is at its highest point. Therefore, when activity is measured under standard conditions, light and dark cycles persist, and activity can be influenced by these light cues. When measured under these cues, activity is measured in zeitgeber time (ZT, "time-giver" in German), as the environmental cues (zeitgebers) of light, dark, and temperature (to name a few) are present to affect behavior (Fig. 1.2; Dunlap et al. 2004; Koukkari and Sothern 2006).

To truly measure endogenously generated circadian activities, rhythms must be measured in the absence of these environmental cues (see Sect. 1.3.1).

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